

- 131 -

Claims

1. An isolated nucleic acid that encodes a CoA ligase, a non-ribosomal peptide synthetase, or a domain of an extender module of a polyketide synthase enzyme that synthesizes FK-520.

5

2. The isolated nucleic acid of claim 1 that encodes an extender module, said module comprising a ketosynthase domain, an acyl transferase domain, and an acyl carrier protein domain.

10

3. The isolated nucleic acid of claim 1 that encodes an open reading frame, said open reading frame comprising coding sequences for two or more extender modules, each extender module comprising a ketosynthase domain, an acyl transferase domain, and an acyl carrier protein domain.

15

4. The isolated nucleic acid of claim 1 that encodes a gene cluster, said gene cluster comprising two or more open reading frames, each of said open reading frames comprising coding sequences for two or more extender modules, each of said extender modules comprising a ketosynthase domain, an acyl transferase domain, and an acyl carrier protein domain.

20

5. The isolated nucleic acid of claim 2, wherein at least one of said domains is a domain of a module of a non-FK-520 polyketide synthase.

6. The isolated nucleic acid of claim 1, wherein said nucleic acid is a recombinant vector capable of replication in or integration into the chromosome of a host cell.

25

7. The isolated nucleic acid of claim 6 that is selected from the group consisting of cosmid pKOS034-120, cosmid pKOS034-124, cosmid pKOS065-M27, and cosmid pKOS065-M21.

30

- 132 -

8. The isolated nucleic acid of claim 5, wherein said non-FK-520 polyketide synthase is rapamycin polyketide synthase, FK-506 polyketide synthase, or erythromycin polyketide synthase.

5

9. A method of preparing a polyketide, said method comprising transforming a host cell with a recombinant DNA vector of claim 6, and culturing said host cell under conditions such that said polyketide synthase is produced and catalyzes synthesis of said polyketide.

10

10. The method of claim 9, wherein said host cell is a *Streptomyces* host cell.

11. The method of claim 9, wherein said polyketide is selected from the group consisting of FK-520, 13-desmethoxy-FK-520, and 13-desmethoxy-FK-506.

15

12. A recombinant host cell that expresses a recombinant polyketide synthase selected from the group consisting of: (i) an FK-520 polyketide synthase in which at least one AT domain is replaced by an AT domain of a non-FK-520 polyketide synthase; (ii) an FK-506 polyketide synthase in which at least one AT domain is replaced by an AT domain of a non-FK-506 polyketide synthase; (iii) an FK-520 polyketide synthase in which at least one DH domain has been deleted; (iv) an FK-506 polyketide synthase in which at least one DH domain has been deleted.

20

13. The recombinant host cell of claim 12 that expresses an FK-520 polyketide synthase in which an AT domain of module 8 has been replaced by an AT domain that binds malonyl CoA, methylmalonyl CoA, or ethylmalonyl CoA.

25

14. The recombinant host cell of claim 12 that expresses an FK-506 polyketide synthase in which an AT domain of module 8 has been replaced by an AT domain that binds malonyl CoA, methylmalonyl CoA, or ethylmalonyl CoA.

30

- 133 -

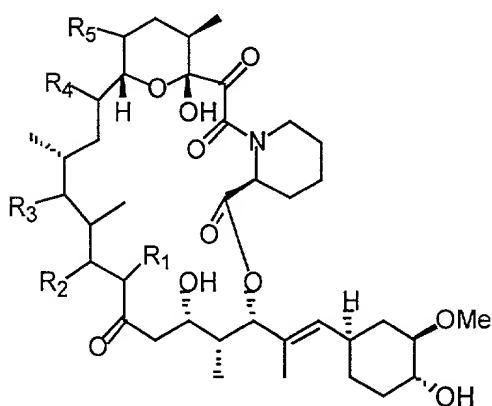
15. The recombinant host cell of claim 13, wherein a DH domain of module 5 or module 6 has been deleted.

5 16. The recombinant host cell of claim 14, wherein a DH domain of module 5 or module 6 has been deleted.

17. A recombinant host cell that comprises recombinant genes coding for enzymes sufficient for synthesis of ethylmalonyl CoA or 2-hydroxymalonyl CoA.

10

18. A polyketide having the structure



15 wherein, R<sub>1</sub> is hydrogen, methyl, ethyl, or allyl; R<sub>2</sub> is hydrogen or hydroxyl, provided that when R<sub>2</sub> is hydrogen, there is a double bond between C-20 and C-19; R<sub>3</sub> is hydrogen or hydroxyl; R<sub>4</sub> is methoxyl, hydrogen, methyl, or ethyl; and R<sub>5</sub> is methoxyl, hydrogen, methyl, or ethyl; but not including FK-506, FK-520, 18-hydroxy-FK-520, and 18-hydroxy-FK-506.

20

19. The polyketide of claim 18 that is 13-desmethoxy-FK-506.

20. The polyketide of claim 18 that is 13-desmethoxy-18-hydroxy-FK-520.